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SHORT COMMUNICATION/KURZMITTEILUNG

Simplification of a Commercially Available Serum Angiotensin-Converting Enzyme Determination

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Summary: The spectrophotometric method for the determination of angiotensin-converting enzyme in serum using *p*-hydroxyhippuryl-*L*-histidyl-*L*-leucine as substrate is commercially available as a test kit. It shows excellent linearity over the whole range of catalytic concentration found in serum. We describe several modifications of this method to simplify and economize the procedure.

Vereinfachung eines kommerziell erhältlichen Tests für Angiotensin Converting Enzyme im Serum

Zusammenfassung: Die spektrophotometrische Methode für die Bestimmung von Angiotensin Converting Enzyme mit *p*-Hydroxyhippuryl-*L*-histidyl-*L*-leucin ist als Testbesteck im Handel. Sie zeigt eine hervorragende Linearität über den gesamten Bereich der in Serum beobachteten katalytischen Konzentration. Wir beschreiben hier mehrere Modifikationen dieser Methode, die das Verfahren vereinfachen und ökonomischer gestalten.

Introduction

In humans, angiotensin-converting enzyme is found in several tissue extracts and fluids.

Angiotensin-converting enzyme is found to be elevated in serum in certain diseases: i. e.: *Gaucher's* disease, hyperthyroidism and sarcoidosis (1). Furthermore sequential measurements of the enzyme in serum are of great help in monitoring the activity of sarcoidosis (1).

Methods have been described for the quantitative determination of the enzyme using the synthetic substrate, hippuryl-*L*-histidyl-*L*-leucine (2, 3). However, these methods are complicated and time-consuming. Recently, *Kasahara & Ashihara* (4) introduced a simple and rapid spectrophotometric method using *p*-hydroxyhippuryl-*L*-histidyl-*L*-leucine as a substrate.

This new method, commercially available as a test kit, shows excellent linearity over the whole range of enzymatic activities found in serum, and a good correlation was found with the spectrophotometric method using hippuryl-*L*-histidyl-*L*-leucine (5). In the present communication several modifications of this method are described, which simplify and economize the procedure.

Materials and Methods

All results obtained with the method under investigation (4) are compared with the results of the fluorimetric assay using hippuryl-*L*-histidyl-*L*-leucine (3). The latter method was performed essentially as described, with the exception that a borate buffer was used instead of a phosphate buffer (6).

The commercially available angiotensin-converting enzyme assay, based on a colorimetric method as described by *Kasahara & Ashihara* (4), was performed according to a modified procedure as described by *Boomsma & Schalekamp* (5). Briefly: to 100 μ l of sample serum, 500 μ l of substrate solution (10 mmol/l *p*-hydroxyhippuryl-*L*-histidyl-*L*-leucine; 2.5 mmol/l 4-amino-antipyrine; 3 kU/l hippuricase in borate buffer) were added. After incubation of this mixture at 37 °C for 30 minutes, 1.5 ml of reaction stopper and developer solution (3 mmol/l EDTA; 6.5 mmol/l NaIO₄; 2 g/l Triton X-100) were added and mixed. Incubation was continued for a further 3 minutes at 37 °C. Serum blanks were prepared by mixing 100 μ l serum with 50 μ l EDTA (40 mmol/l) before addition of the substrate solution. The final concentration of EDTA is sufficient for complete inhibition of the catalytic activity (4).

By pipetting the amounts of serum and reagents as recommended by the manufacturer, one packaged assay kit serves for the assay of 20 samples.

We have modified this procedure in such a way that 100 instead of 20 samples can be assayed. Using the above-mentioned procedure the quantity of sample serum is reduced from 100 μ l to 20 μ l. To keep the protocol unaltered the quantities of the successive reagents are also reduced by the same factor. The final reaction volume becomes 420 μ l. After the last incubation step, 250 μ l of the 420 μ l is pipetted into the wells of a microtiter plate and absorbance is measured in a Titertek® Multiskan photometer (Flow Laboratories, Ltd Scotland) at 492 nm.

The catalytic concentration was calculated according to the formula.

$$\frac{A_{\text{sample}} - A_{\text{blank}}}{12000} \times \frac{0.42}{0.02} \times \frac{1}{30} \times 10^6 \times 1.45 [\mu\text{mol}/\text{min} \cdot \text{l}], [\text{U}/\text{l}]$$

The factor 1.45 has been introduced to correct for the measurement at a wavelength of 492 nm instead of 505 nm and for the shorter optical path length of the sample in the microtiter plate.

As recommended by the manufacturer, the reconstituted substrate should be used within a day and the reconstituted stopper and developer substance within 7 days when stored at 4 °C. To test the stability of the reagents after reconstitution when stored at -70 °C, aliquots of the reagents were frozen in polystyrene tubes immediately after reconstitution. After one to eight weeks reagents were thawed and used in the test.

Results and Discussion

Results obtained with the modified method by using small volumes and microtiter plates and the method of Friedland & Silverstein (3) were compared. With 44 different sera a very good correlation between the fluorimetric method (x) and the present method (y) was found (fig. 1). The linear regression equation calculated was $y = 0.34x + 0.54$ with $r = 0.981$. Two sera were used to determine the interassay variation as well as the intra-assay variation. As shown in table 1, small coefficients of variation were found. Moreover performing the test in duplicate did not increase precision. It was observed that storage of reconstituted reagents at -70 °C for a period up to 8 weeks did not result in any observable loss of reactivity. Test results obtained by using reconstituted and stored reagents were identical with those obtained with freshly reconstituted reagents. Reagents that are not used within a day can therefore be stored at -70 °C and used later.

From the results it is clear that the angiotensin-converting enzyme activity can be accurately measured with the proposed modifications of the test kit. With these modifications the method is partly automated and becomes more economical, permitting a 5-fold increase in the number of possible assays.

References

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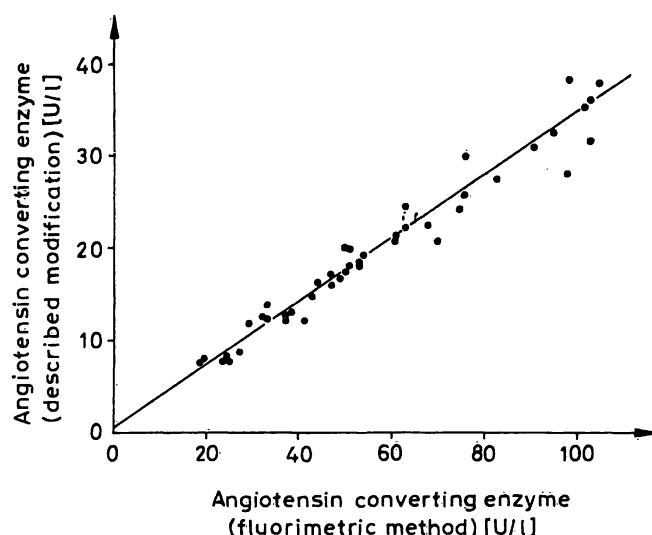


Fig. 1. Relation between the fluorimetric method (substrate: hippuryl-L-histidyl-L-leucine) and the colorimetric test kit modified as described (substrate: *p*-hydroxyhippuryl-L-histidyl-L-leucine). The linear regression equation was $y = 0.34x + 0.54$ with a correlation coefficient of 0.981.

The catalytic concentration of angiotensin-converting enzyme was measured in 44 sera.

Tab. 1. Interassay variation and intra-assay variation of the modified method for angiotensin-converting enzyme catalytic concentration.

	Se- rum	N	Catalytic concentration		CV (%)
			\bar{x} (U/l)	SD (U/l)	
Interassay (singular)	A	17	19.0	1.3	6.9
	B	17	14.7	0.9	6.1
Interassay (duplicate)	A	18	18.8	1.3	6.9
	B	18	14.6	1.0	6.5
Intra-assay (singular)	C	14	19.8	0.9	4.6

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